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THE TECHNIC OF THE WASSERMANN REACTION

WITH REFERENCE TO THOMAS AND IVY'S METHOD OF COMPLEMENT DOSAGE AND TO THE MANAGEMENT OF NATURAL ANTISHEEP AMBOCEPTOR*

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Thomas and Ivy[1] have recently proposed a method for increasing the delicacy of the Wassermann reaction. Their method consists of the use of the minimum amount of complement and amboceptor which will give complete hemolysis in the presence of the proper quantity of antigen and of (pooled) negative serum. They make no reference to the occurrence of natural antisheep amboceptor in the human serum which was added in titrating the complement. It attempting to test their method we have found that this may occasionally lead to error.

Many authors have referred to the frequent occurrence of natural antisheep amboceptor in appreciable amounts in human serum. The well-known modifications of Bauer,[2] as well as those of Hecht and Weinberg and others, take advantage of this and the substitutes for the Wassermann method, such as that of Noguchi, are devised chiefly to eliminate the error caused by it. The occasional occurrence of a great excess of such natural amboceptor is also well recognized.[3] We have found in 2,158 tests that 955 sera (44 percent) had an appreciable amount of natural amboceptor and that 476 sera (21 percent) had sufficient to produce complete laking without further addition of amboceptor.

Thus, a number of negative sera pooled at random almost always have some natural amboceptor, and we have occasionally found that such pooled sera had so much natural amboceptor that complete hemolysis occurred without the addition of artificial amboceptor. It is obvious (from the law of inverse ratios of Morgenroth and Sachs[4]) that under these circumstances the complement titration would give too

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[1] Am. Jour. Med. Sc., 1914, 148, p. 55.
[2] Berl. klin. Wchnschr., 1908, 15, p. 843.
[3] Kaliski: Arch. Int. Med., 1910, 6, p. 205.
[4] Berl. klin. Wchnschr., 1902, 39, p. 817.

small a dose. If Thomas and Ivy's proposal is adopted, it is therefore necessary in every case to choose negative sera which are known to have little or no natural amboceptor.

Altho some workers pay no attention to natural amboceptor, a perusal of the literature and our own experience leave us no doubt that if the presence of natural amboceptor is disregarded a certain small percentage of false negative reactions is obtained. Some authors have undoubtedly exaggerated the proportion of such cases. Nevertheless, it is obvious that the occasional coincidence of a large excess of natural amboceptor with a small amount of so-called "syphilitic antibody" will inevitably lead to error. In spite of the many methods which have been proposed to obviate this difficulty there is none entirely satisfactory. The absorption methods are accurate but cumbersome and sometimes render sera anticomplementary (Sachs phenomenon). Some observers[5] make a preliminary test, often called the "Bauer control tube," to determine the natural amboceptor and then add immune amboceptor only to those sera that need it.

The method we have adopted, which originated at Mount Sinai Hospital with Dr. D. J. Kaliski,[6] is similar to the Bauer method, but simpler. It consists of the addition of non-sensitized sheep cells, after the preliminary incubation, to all the given tests and incubation for ten minutes in the water bath at 37.5 C. At the end of this time those tests which contain a considerable excess of natural amboceptor show complete laking in the control tubes,[†] and to these tests no amboceptor is added. To all the other tests, the usual amount of two units of amboceptor is then added, and all the tests are incubated for the full period. Thus, the addition of still more amboceptor to those tests that already contain an excess is avoided.

It must be borne in mind that even with this method those rather exceptional sera that have a very great excess of natural amboceptor may still give false negative results. These sera (recognized by rapid laking in all the tubes) are re-tested after absorption of the natural amboceptor.[7]

Some workers from the New York Department of Health attempt to surmount the difficulty by reading the results as soon as the antigen and serum controls are laked. Thus, Olmstead[8] says: "An excess of amboceptor may result in false negative reactions. If, however, the readings are made as soon as antigen and serum controls are completely hemolyzed, an excess of amboceptor makes little difference in results."

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[5] Dexter and Cummer: Arch. Int. Med., 1912, 9, p. 605.

[6] Arch. Int. Med., 1912, 9, p. 214.

[†] Two control tubes are used for each serum, the one containing a full dose of serum (0.1) the other a half dose (0.05). The presence of two or more units of natural amboceptor is shown by complete laking in both of these tubes.

[7] Rossi: Ztschr. f. Immunitäts., 1911, 10, p. 321; Jacobaeus, Ibid., 1911, 8, 615.

Our experience, however, has shown us that those sera which contain an excess of natural amboceptor always lake much more rapidly than the antigen control, which of course contains only two units of amboceptor.

CONCLUSIONS

If attention is not paid to natural amboceptor a certain, tho small, percentage of positive results will be overlooked.

If Thomas and Ivy's method of titrating complement is used, it is essential to select negative sera which have no natural amboceptor.

Aside from this criticism, we shall not attempt here to pass judgment on Thomas and Ivy's method, but our experience leads us to fear that, if their proposal to use a single laking dose of complement and amboceptor is adopted, the margin of safety may not be large enough to avoid occasional, non-specific complement fixation.

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[8] Med. Rec., New York, 1914, 85, p. 341.